REMARKS

A. Request for Reconsideration

Applicant has carefully considered the matters raised by the Examiner in the outstanding Office Action but remains of the position that patentable subject matter is present. Applicant respectfully requests reconsideration of the Examiner's position based on the amendments to the claims and the following remarks.

B. The Invention

The present invention is directed to a process for the separation, identification and quantification of individual microbes in a mixture of microbes using electrokinetic separation systems.

In one of the novel aspects of the invention, similar types of microbes are focused in a moving fluid with a dilute water soluble neutral polymer. The focusing effect resulted from the adding a dilute hydrophilic neutral polymer in the present invention is evident from the sharp peaks in an electropherogram.

C. Claim Status and Amendments

Claims 1, 3-6, 8-13, 15, 17 and 22-30 are presented for further prosecution. Claims 18-21 have been withdrawn from consideration.

Claim 1 and claim 4 have been amended by following the examiner's suggestion on the informalities. Namely, "microbes" in lines 2 and 17 of Claim 1 have been changed into "microbes/cells"; "analysis' in claim 4 has been changed into "analyzing".

Claims 1 6, 10, 15 and 30 have been amended to recite that the microbes/cells are separated by electrophoresis using a dilute water soluble neutral polymer in the moving fluid. Support for this amendment can be found in the examples 1-6 and 8-12.

Claims 22-25 have been amended to emphasize that the dilute water soluble polymer is neutral instead of being charged. The Support for this amendment is the fact that all the polymers numerated in the page 14, paragraph 4 of the specification and claims 22-25 are neutral polymers.

Claim 26 has been amended back into previous wording to recite the capillary isoelectric focusing technology. The support for this amendment is in the example 7 of the specification.

D. <u>The Office Action</u>

1. Claims 27-29 had been allowed and the restoration of the allowed status is respectfully requested.

Claims 27-29 had been allowed in the previous Office Action dated August 2, 2005 (page 20-21). In the present office action claims 27-29 had been rejected under USC 112 1st paragraph as "require performing isoelctric focusing on sample in a moving fluid" (See page 3 of the Office Action). This rejection is not applicable to Claims 27-29, since claims 27-29 relate to conventional capillary isoelectric focusing technique without the requirement of a "moving fluid". It is respectfully requested that the allowed status of the claims 27-29 be restored.

2. Independent claims 1 6, 10, 15 and 30 and their dependent claims as currently amended are clear of 35 USC 112 paragraph 1 and 2 rejections

Regarding the USC 112 1st paragraph rejection, independent claims 1 6, 10, 15 and 30 have been amended to recite that the microbes/cells are separated by electrophoresis in a moving fluid. By amending "isoelectric focusing" to "separating" by "electrophoresis", these claims are consistent with the

electrokinetic feature of the electrophoresis technique, where the fluid in the separation passageway is "moving" due to the electroosmotic flow.

As for USC 112 2nd paragraph rejection, independent claims 1 6, 10, 15 and 30 have also been amended by delete "means of an electric field" to emphasize the focusing effect due to the using of dilute water soluble neutral polymers. The current wording recites that the microbes/cells are separated by electrophoresis by using a dilute water soluble neutral polymer to focus them together. The innovative use of the diluted neutral polymers in the running buffer focuses the microbes/cells into a compact zone. This obvious focusing effect can been seen in the electropherograms as very sharp peaks.

Claims 1 6, 10, 15 and 30 and Claims 22-25 have also been amended to emphasize that the dilute water soluble polymer is neutral instead of being charged. All the polymers numerated in the specification and claims are neutral polymers. The diluted water soluble neutral polymer in the running buffer exerts a focusing effect by different mechanism from conventional usage of concentrated, charged polymers in the electrophoresis methods.

It should be clear that, by reciting the focusing effect of the suitable diluted water soluble neutral polymers in the running buffer of a typical electroporesis method, claims 1, 6, 10, 15, and 30, along with their dependent claims 3-5, 8-9, 11-13, 17 and 22-25 comply with the writer description requirement of USC 112 1st paragraph and are definite according to USC 112 2nd paragraph.

4. Prior Art Rejection under USC 103

Claims 26 and 30 had been rejected as unpatentable over Fuhr; and claim 27 had been rejected as unpatentable over Fuhr in view of Durr and Tollet.

Fuhr is the primary reference and the only reference that had been cited to teach isoelectric focusing step of the claims 26, 27 and 30.

As stated above, Claim 30 has been amended to recite that the microbes/cells are separated by electrophoresis using a dilute water soluble neutral polymer in the moving fluid. Fuhr does not teach such a step, therefore the USC 103 rejection on Cliam 30 over Fuhr is moot.

Claims 26 and 27 specifically recite capillary isoelectric focusing technology is used in separation step. Fuhr uses a different technology compared with the typical capillary isoelectric focusing (CIEF). These differences have the following aspects:

1) Fuhr uses an external field (See col. 5, lines 38-40); CIEF uses an "internal" field with direct electrical contact with the buffer and with current flowing in the separation channel.

2. In Fuhr, the external field is perpendicular to the channel and the flow direction (see Fig. 3); In CIEF the current and field are parallel to the channel.

In fact, the external perpendicular field in Fuhr is totally uncompatible with the "internal" parallel electric field so that it can not generate a pH gradient along the capillary channel, which is the essence of CIEF. A summary introduction on CIEF is attached for your reference. The Figure 25 shows the electric filed applied along the capillary.

Applicants respectfully submit that Fuhr is in a different technology from the capillary isoelectric focusing used in Claims 26 and 27 and it doesn't teach the elements of the

present invention as in claims 26 and 27. It is therefore believed that the present invention is patentable over Fuhr.

E. Conclusion

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance and such action is respectfully requested. Should any extensions of time or fees be necessary in order to maintain this Application in pending condition, appropriate requests are hereby made and authorization is given to debit Account # 02-2275.

Respectfully submitted,

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Encl: Webpages of Chemsoc.org on Isoelectric Focusing

Return-receipt postcard

Isoelectric points

Theory
Modes
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Some molecules contain both acidic and basic groups, these are known as zwitterions. At a particular pH the charge on the groups is balanced and the molecules are neutral. This is known as their isoelectric point. If a zwitterion is placed in a pH gradient and electrophoresed it will migrate to the point at which it is uncharged and then stop moving. This is the basis of capillary isoelectric focusing (cIEF).

A pH gradient is formed in the capillary by filling it with a solution of molecules known as ampholytes. These molecules are also zwitterions prepared with pI's across a broad range. Thus when an electric field is applied these will form a pH gradient. After filling the capillary with a mixture of solute and ampholytes the gradient is formed. With a basic solution at the cathode, and an acidic one at the anode, upon application of an electric field the charged ampholytes and proteins migrate through the medium until the reach a region where they become uncharged (at their pI). This process is known as 'focusing'. The protein zones remain narrow since a protein which migrates out of it zone will become charged and migrate back.

The following movie illustrates the process. **Click the image** to see it.

Figure 25: Isoelectric focusing

You can view another movie of the process by clicking here (Realmedia).

The focusing process

Once focusing is complete a steady state is reached where no current flows through the capillary. At this point there are no charged molecules, hence nothing to carry the current. To observe the analytes the zones are mobilised and passed through the detector. Mobilisation is accomplished by application of pressure to the capillary or addition of salt to one of the reservoirs.

The EOF needs to be eliminate in cIEF as the flow would flush the ampholytes from the capillary before focusing was complete. In order to reduce the EOF dynamic or covalent coatings are used as described in the wall section.

As the protein is loaded into the capillary during filling it is possible to load large volumes compared to other CE modes. Precipitation, which results from very high protein concentrations within the zones, is usually the limiting factor in sample loading.

The result of a capillary IEF separation is shown in the following figure and in references 6 & 7.

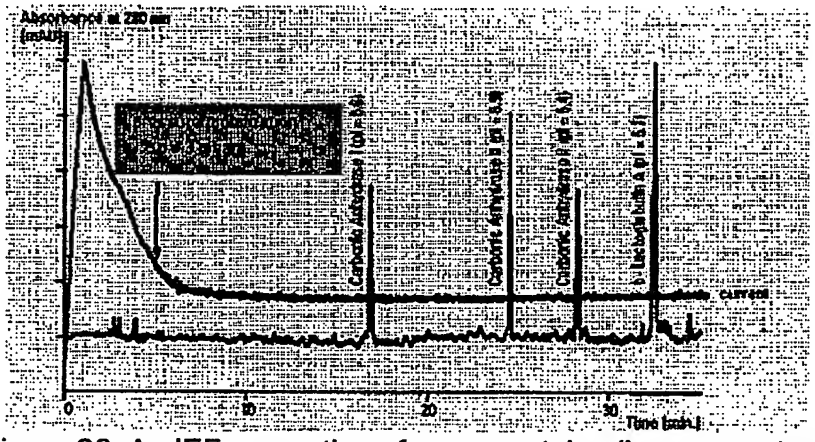

Figure 26: An IEF separation of some proteins (image courtesy of Agilent Technologies)